DECOMPOSITION OF GUAYULE RESINS BY MICROORGANISMS[1]

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INTRODUCTION

A process for the aerobic biological decomposition of guayule (*Parthenium argentatum* Gray) prior to extraction of the rubber by mechanical means was patented by Spence (1933). The process, called retting because of the loose analogy to the retting of flax for the production of linen fiber, produced a marked decrease in the impurities of the rubber and improved the physical properties of the vulcanized rubber. It also decreased the resinous acetone-soluble contaminant from about 20 per cent of the crude rubber product to about 10 per cent, but no conclusions were drawn as to the agent responsible for the changes in composition and properties. The work reported in this paper is one phase of a reinvestigation of the retting of guayule by the natural microflora; it deals specifically with the decomposition of guayule resins. The resin of guayule is a mixture of acetone-soluble constituents of the plant. When the shrub is injured a clear resin exudes and dries. Other resin preparations were made by exhaustive extraction of guayule rubber with acetone and removal of the solvent.

A variety of microorganisms normally present in the soil flora have been shown to oxidize compounds ordinarily considered relatively resistant to biochemical oxidation. Numerous species of bacteria have been found to oxidize phenols, and aromatic, alicyclic and aliphatic hydrocarbons. The literature on these subjects prior to 1940 is briefly reviewed by Bushnell and Haas (1941). Many species of actinomycetes oxidize aliphatic hydrocarbons (Umbreit, 1939) and the olefinic rubber hydrocarbon (Spence and Van Niel, 1936; Kalinenko, 1938, 1940; ZoBell and Grant, 1942). Fungi, especially species of *Aspergillus* and *Penicillium*, have also been shown to oxidize aliphatic hydrocarbons (Rahn, 1906) and rubber (Söhngen, 1906; Söhngen and Fol, 1914; Kalinenko, 1938, 1940). Results of detailed analysis of the microbiological and chemical changes and a description of the conditions prevailing during retting are presented in another paper (Naghski, White, and Hoover, 1944).

PROCEDURE

Methods of studying the decomposition of water-insoluble compounds

In the microbiological decomposition of water-insoluble compounds, one of the major difficulties encountered by both the organism and the investigator is ob-

[1] Natural Rubber from Domestic Sources. Paper No. 2.

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taining an intimate contact between the substrate and the water phase which must serve as a culture medium for the organism. Although complete molecular dispersion of such compounds cannot be attained, it can be approached by emulsification of the substrate in the water medium.

The increased area of interface will facilitate decomposition, whether actual contact between bacteria and substrate is necessary or whether it is only necessary that bacterial enzymes should have access to the substrate. If water-soluble end products are formed, the resultant clearing of the emulsion will afford a visual criterion of decomposition. This technique was successfully used by Eijkman (1901) in his studies on starch- and protein-decomposing enzymes in bacteria. With modifications, such as the inclusion of dyes which change color in the presence of decomposition products (Collins and Hammer, 1934), it is now commonly used in the study of lipase and amylase production by bacteria. Eijkman pointed out that clearing of the emulsion beyond the boundaries of a bacterial colony is good evidence of the production of extra-cellular enzymes responsible for the decomposition. Spence and Van Niel (1936) used the same principle in a study of rubber-decomposing organisms, and were able to demonstrate a clearing of the opaque latex of *Hevea* by several strains of *Actinomyces*.

Waksman and Hutchings (1936) employed an emulsion of phenol and lignin in water to study lignin digestion by bacteria and fungi. The digestion of the substrate was demonstrated by recovery of the residual lignin. In the work reported here, an analogous method was employed to determine quantitatively the disappearance of resin in a liquid culture.

Preparation of resin emulsions

The procedure for preparing emulsions was as follows: Resin was dissolved in acetone to give a 2-per cent solution. Twenty ml of this was poured into 1 liter of half strength Allison's solution (Allison and Hoover, 1934) at 90 C with rapid stirring, giving an emulsion containing 0.4 gm of resin per liter. The acetone was then driven off by heating on a steam bath for several hours. The resulting emulsion was used as a basic medium to which mineral salts and other nutrients were added as desired. When the pH was properly adjusted, it was stable to autoclaving and could be kept indefinitely without added agar. In liquid media, the emulsions used were stable in the pH range of 6.5 to 11.0 but unstable at lower pH values. The pH of all media, therefore, was kept between 6.5 and 7.0.

By using more of the 2-per cent acetone solution of resin, we obtained more concentrated emulsions. Up to 1.5 gm of resin per liter, emulsification was complete, but as the concentration was increased beyond this amount, more and more of the resin either failed to emulsify or quickly agglomerated and floated out as large drops. Variations in particle size were obtained by varying the concentration of the acetone solution. A finer emulsion was prepared with a 1-per cent solution than with a 2-per cent solution. The resulting advantage of having fine particles was partly counterbalanced by the agglomeration which occurred during the prolonged heating necessary to drive off the greater quantity of acetone present.

RESULTS

Isolation of organisms clearing resin emulsions

Samples of the retting shrub were cut aseptically into fine pieces. Ten grams were suspended in 90 ml of sterile water and allowed to stand for half an hour, then shaken vigorously, and plated out according to standard bacteriological practice. The medium used for plating was a resin emulsion, mineral-salts agar of the following composition:

Medium I

Resin emulsion	100 ml.
NH_4NO_3	0.01 gm.
Agar	2.0 gm.

Poured plates were incubated at 30 C for at least 21 days, unless otherwise specified, and examined at intervals of 1 to 3 days. Colonies which affected the resins were located by examining plates in reflected light against a dark background. Some of the colonies which cleared the resin emulsion were picked and streaked on Medium I, from which single-colony isolates of resin-clearing organisms were made. Each isolate was subsequently transferred to a resin-emulsion nutrient agar of the following composition:

Medium II

Medium I	100 ml.
Beef extract	0.3 gm.
Peptone	1.0 gm.

Pure cultures of several types of resin-clearing organisms were also obtained by dilution and plating out of a small piece of agar containing a resin-clearing colony. The agar was suspended in sterile distilled water for several hours, macerated with a needle, and occasionally shaken vigorously. It was then plated out in Medium II, from which single-colony isolates were made.

Organisms which did not clear resin emulsions developed on both Medium I and II. At the end of 3 days' incubation, the total count on Medium II corresponded closely to the total count obtained from cultures plated out in beef-extract peptone agar. On Medium I colonies which did not clear the resin emulsion developed slowly, but if the plates were kept for several weeks, counts were obtained which also agreed well with those on Medium II and on beef-extract peptone agar. In the concentrations which we used, the resin is, therefore, not appreciably bactericidal.

Microscopic examination showed that resin particles less than 1 micron in diameter, which were numerous in uncleared emulsions, had completely disappeared in cleared emulsions. Larger particles, up to 10 microns in diameter, had become irregular in outline, sometimes fragmented, and appeared to be of heterogeneous composition. There was no evidence of aggregation or agglomeration of particles. Analyses of liquid emulsions showed that there was a marked loss of water-insoluble, acetone-soluble material during the growth of

resin-clearing organisms but not during the growth of organisms which caused no clearing (Table III). The clearing of resin emulsions in agar is, therefore, a reliable criterion of resin decomposition.

Organisms isolated

The ability to clear emulsions of guayule resin is possessed by a great variety of microorganisms found on retting shrub. Isolates were made of the predominating types of resin digesters, and the organisms were identified as three genera of fungi, one actinomycete, and five genera of bacteria.

A. *Fungi.* The most common resin-clearing fungus (1HF, Plate II, 0) is *Aspergillus fumigatus.*[3] It appeared on retting shrub in counts as high as 500,000 per gram, and after the end of the first week was the predominating type of fungus. It grew well on ordinary nutrient media and tolerated temperatures as high as 50 C, but did not grow at 55 C. Clearing of the resin in Medium I appeared on the second day after inoculation, when the colonies were about 5 mm in diameter, and thenceforth was co-extensive with the mycelium.

A second fungus (4HF) isolated from retting shrub cleared resin emulsions in 3 to 4 days. On the basis of cultural and morphological characters it was readily identified as *Rhizopus arrhizus* (Henrici, 1930). Our isolate grew at 38 C but not at 45 C.

Another fungus (3HF) was isolated from shrub that had been retting 17 days. On the basis of morphological characteristics it was placed in the Dematiaceae.[3] It was a relatively slow-growing organism. The temperature for optimum growth was 37 C, and that for maximum growth was 42 C. On Medium I it produced distinct clearing of the resin emulsion in 2 days at 37 C. (Plate I, E). The zone of clearing extended slightly beyond the edge of the colony. This organism cleared resin emulsion agar more completely than any ot the other organisms isolated.

B. *Actinomycetes.* During the third week the retting shrub became densely covered with actinomycetes. One type (1HA) showed resin clearing (Plate I, D and H) in a culture four days old. It was isolated, and its cultural and biochemical reactions were studied on differential media. Mycelium occurred as branched filaments without spirals. No soluble pigment was produced. Nutrient agar colonies had grayish aerial mycelium with yellow-orange reverse color. The reverse color was purplish black on calcium malate agar and yellow on potato and on protein-glycerol agars. It hydrolyzed starch; peptonized litmus milk slowly; liquefied gelatin in 7 days; made glucose broth acid, changing to alkaline; and made lactose broth alkaline. The temperature for optimum growth was 28 to 42 C; for maximum growth it was 50 C. On the basis of these characteristics, the organism was identified as *Actinomyces fradii* Waksman and Curtis.

C. *Bacteria.* Bacteria capable of clearing resin emulsions made up from 0.5 to 1.0 per cent of the total number of microorganisms on the retting shrub, reaching maximum counts of from 5 to 6 million per gram. Isolates from routine

[3] We are indebted to Dr. K. B. Raper, Fermentation Division, Northern Regional Research Laboratory, for the identification of these organisms.

plating were separated on the basis of gram morphology into three groups, one of which was further subdivided culturally, as indicated in the following key:

Group I. Gram-positive cocci (1 isolate)..... (*Micrococcus*)
Group II. Gram-positive sporeformers (3 isolates)..... *Bacillus subtilis*
Group III. Gram-negative rods (9 isolates)
1. Not growing on any ordinary media (2 isolates)..... (*unidentified*)
2. Growing well on all ordinary media
(a) Motile (4 isolates)..... *Pseudomonas boreopolis*
(b) Nonmotile (3 isolates)..... *Achromobacter lacticum*

Group I—Gram-positive cocci (11H). Cells occurred singly or in pairs, and occasionally in chains of 3 to 6; did not form tetrads; were 0.7 to 1.0 micron in size; and required organic nitrogen for growth. Nutrient agar colonies were white, smooth, entire, with centers turning slightly brown on aging. The organism caused infundibuliform liquefaction of gelatin by the eighth day; acid-coagulated milk, with reduction of litmus; produced acid from glucose and lactose; reduced nitrates to nitrites; did not produce indole; and hydrolyzed starch. Growth occurred at 45 C but not at 50 C. This organism probably belongs to the genus *Micrococcus*. Although it is not concluded that this culture represents a species hitherto not described, the schemes of classification of Bergey (1939) and of Lehmann and Neumann (1931) offer little help in its identification.

Group II—Gram-positive spore-forming rods (9H, 10H, 12H). All isolates capable of attacking resin belonged to the *Bacillus subtilis* Cohn group, although certain strains of *B. subtilis* were isolated which lacked this ability. Unlike the gram-negative resin-clearing bacteria, they required an organic source of nitrogen for active resin decomposition.

Group III—1. Gram-negative rods not growing on ordinary media (1H). The rods were short (0.5 to 0.7 x 1.0 to 2.0 microns) and nonmotile. The organism did not grow on ordinary laboratory media, but grew on media containing resin as a source of carbon, with inorganic or organic nitrogen. At best, growth was slight. Resin-agar colonies were small (0.5 to 1.5 mm), convex, smooth, with yellow pigmentation. The temperature for optimum growth was 21 C; the maximum temperature for growth was from 33 to 37 C.

Group III—2a. *Pseudomonas* sp. (3H, 8H, 16H, 17H). Short rods (0.5 to 0.8 x 1.5 to 2.5 microns) occurring singly and in pairs (certain strains produced filaments), motile, with 2 to 4 polar flagella. Growth on nutrient agar slant was yellowish (occasionally tinged with brown) and filiform. Agar colonies were circular, smooth, and yellowish. The organism liquefied gelatin slowly (one strain failed to liquefy within 21 days). It coagulated litmus milk, with complete reduction of litmus, followed by slow peptonization. It produced a slight acidity in glucose and lactose broths, changing to alkaline. It did not hydrolyze starch; sometimes it reduced nitrates to nitrites, and sometimes it did not; and it did not produce indole. Growth occurred at 50 C but not at 55 C. This description agrees with the description of *P. boreopolis* Gray and Thornton in Bergey's manual.

Group III—2b. *Achromobacter* sp. (4H, 6H, 13H). Short, coccoid rods (0.9 to 1.6 x 0.9 to 2.5 microns), nonmotile. Nutrient agar colonies were white and convex. Growth on agar slant was white and filiform. The organism did not liquefy gelatin; it acid-coagulated litmus milk, with reduction of litmus; it produced acid from glucose and lactose; it did not hydrolyze starch; it did not reduce nitrates to nitrites; and it did not produce indole. Growth occurred at 50 C but not at 55 C. These organisms fit the description of *A. lacticum* (Kramer) Bergey, *et al.*

Behavior toward different resins and resin fractions in Medium I

The microbiological decomposition of resins from different sources and of some of the isolated components of these resins (Table I) was studied, the agar emulsion technique being used. The alcohol, ester, and wax were pure compounds isolated by E. D. Walter of this Laboratory.

TABLE I

Resin preparations

RESINOUS MATERIAL	SOURCE AND NATURE
Resin #1	Unsaponifiable fraction of leaf or crude rubber extract
Resin #2	Whole acetone extract of dried guayule leaves
Resin #3	Whole acetone extract of crude guayule rubber
Resin #4	Exuded resin picked from dried shrub
Sesquiterpene alcohol	Isolated in pure form from resins 2 and 4
Cinnamic acid ester of the sesquiterpene alcohol	Present in resins 2, 3, and 4, particularly 4
Wax	Present in resins 2 and 3 but not in 4

The exuded resin contained volatile oils and approximately 20 per cent of the cinnamic acid ester of the sesquiterpene alcohol, in addition to other undetermined constituents. The acetone extracts contained the same components as the exuded resin, plus wax, carotenoids, chlorophyll, and possibly flavones (Walter, 1944).

Table II gives a summary of the clearing of emulsions of these resins. Clearing in Medium I containing resin 2 or 3 was produced within a week by all except the *Micrococcus* and the *B. subtilis* isolates. The *Micrococcus* did not grow at all on this medium, and *B. subtilis* grew very slowly. After six weeks, however, the colonies of *B. subtilis* were about 2 mm in diameter and showed very faint signs of clearing.

The unsaponifiable fraction was affected only by 1H (unidentified rod), 3HF (the member of the Dematiaceae), and *A. fradii*. 3HF produced distinct clearing in 3 to 4 days, but 1H (unidentified rod) and *A. fradii* required about 10 days. The exuded resin was attacked only by 1H (Plate I, A) and 3HF. The wax, alcohol, and ester were not affected by any of these organisms even after prolonged incubation, although all the organisms which produced clearing of resin emulsions will grow on media containing these compounds.

The rapidity and extent of emulsion clearing varied with the organism. If growth was rapid, as in the case of *A. fumigatus*, clearing never extended beyond the borders of the colony, but if growth was restricted, as in the case of submerged bacterial colonies, clearing extended beyond the limits of the colony (Plate II). The decomposition of resin, therefore, must be accomplished by substances which diffuse out from the bacterial or fungus cells. Oxidation of the resin, either completely to CO_2 and H_2O, or partly to compounds even slightly soluble in water, would result in the disappearance of resin particles. Emulsion clearing may therefore be caused by an oxidative decomposition catalyzed by extra-cellular enzymes. The isolation of such enzymes has not been attempted.

TABLE II

Clearing of different resins at 30 C*

CULTURE	ORGANISM	MEDIUM I				MEDIUM II		
		Resin 2 (leaf extract)	Resin 3 (crude rubber extract)	Resin 1 (unsaponifiable)	Resin 4 (exuded resin)	Resin 3	Resin 1	Resin 4
	Bacteria							
1H	Unidentified rod	++	++	++	++	++	−	−
3H	*Pseudomonas boreopolis*	++	++	0	0	++	0	0
8H	*Pseudomonas boreopolis*	−	+	0	0	++	0	0
4H	*Achromobacter lacticum*	−	++	0	0	++	0	0
6H	*Achromobacter lacticum*	−	++	0	0	++	0	0
9H,10H,12H	*Bacillus subtilis*	−	±	0	0	++	0	0
11H	*Micrococcus sp.*	0	0	0	0	++	0	0
	Fungi							
1HF	*Aspergillus fumigatus*	++	++	0	0	++	0	0
3HF	Dematiaceae	−	+++	+++	+++	+++	+++	+++
4HF	*Rhizopus arrhizus*	−	++	−	−	++	−	−
	Actinomycete							
1HA	*Actinomyces fradii*	++	++	+	0	++	++	0

* The amount of emulsion clearing is indicated by symbols, from ++++, complete clearing, to 0, no clearing. Cases not studied are indicated by −.

Effect of added nutrients on resin decomposition

The presence of organic nitrogen compounds and of other organic compounds besides resin did not prevent the clearing of resin emulsions (Table II). In fact, on media containing peptone and beef extract (Medium II), clearing in some cases appeared more quickly, apparently because of the more rapid growth. 3HF and 1H grew as well on Medium I as on Medium II and produced about the same clearing. The other organisms, however, showed some notable differences between the resin clearing in Medium I and that in Medium II. These differences were primarily correlated with differences in growth rate. Thus the *Micrococcus* produced no growth and hence no clearing in Medium I, and *B. subtilis*, growing slowly on Medium I, was much slower in producing resin clearing on this medium than on Medium II, where growth was luxuriant. Similarly for other

isolates, the rate and extent of clearing of crude-rubber resin were correlated with the amount of growth.

With the exception of 1H (unidentified rod), which grew slowly on any medium, the microorganisms studied did not grow as well with resin as their sole organic substrate as they did when supplied with beef extract and peptone. Either this

TABLE III

Loss of resin 3 after incubation at 30 C, as determined by chloroform extraction of the liquid emulsion

CULTURE	ORGANISM	MEDIUM	AGE OF CULTURE	RESIN			
				Control flask	Inoculated flask	Loss	Loss per day
			days	*mg*	*mg*	%	%
	Bacteria						
1H‡	Unidentified rod	I—100 ml	6	94.0	81.0	14.0	2.3
1H‡	Unidentified rod	I—100 ml	19	86.0	48.0	44.0	2.3
3H	*Pseudomonas boreopolis*	I—100 ml, II—10 ml	12	56.3*	36.5	35.0	2.9
8H	*Pseudomonas boreopolis*	I—100 ml, II—10 ml	12	56.3*	36.7	35.0	2.9
4H	*Achromobacter lacticum*	I—100 ml	14	47.1†	40.3	14.5	1.0
4H	*Achromobacter lacticum*	I—100 ml, II—10 ml	12	56.3*	38.4	32.0	2.7
6H	*Achromobacter lacticum*	I—100 ml, II—10 ml	12	56.3*	36.0	36.0	3.0
9H	*Bacillus subtilis*	I—100 ml, II—10 ml	12	56.3*	51.5	8.5	0.7
9H	*Bacillus subtilis*	I—100 ml	21	47.1†	43.6	7.5	0.4
166‡	Unidentified rod	I—100 ml	19	62.0	64.0	−3.0	—
	Actinomycetes						
1HA	*Actinomyces fradii*	I—100 ml	17	47.1†	32.7	30.5	1.8
	Fungi						
1HF	*Aspergillus fumigatus*	I—100 ml	16	47.1†	29.3	38.0	2.4
1HF	*Aspergillus fumigatus*	I—100 ml, II—10 ml	7	56.3*	44.8	20.5	2.9
3HF	Dematiaceae	I—100 ml, II—10 ml	12	56.3*	47.0	16.5	1.4
3HF	Dematiaceae	II—100 ml	13	50.7	32.1	36.7	2.8

* Average of 2 control analyses, which gave 53.5 and 59.1 mg, respectively.
† Average of 3 control analyses, which gave 48.9, 47.0, and 45.4 mg, respectively.
‡ Acid-ether extraction and resin 2 were used.

simple medium did not supply accessory growth substances required or the rate at which resin was decomposed was not rapid enough to support optimum growth.

Disappearance of resins from liquid-emulsion cultures

Quantitative determinations were made of the amount of resin lost from liquid emulsions (Medium I without the agar) during the growth of resin-clearing organisms. One hundred- or one hundred and ten-milliliter aliquots were pipetted

into 250-ml Erlenmeyer flasks, sterilized, inoculated, and incubated at 30 C for 6 to 21 days. The residual resin was then recovered by acid-ether extraction or by refluxing with chloroform. The water emulsion of chloroform obtained in the second case was broken by centrifugation. The resulting extracts were washed with distilled water and evaporated to dryness, and the recovered resin was weighed. The recovered product was in all cases completely soluble in acetone. The results, presented in Table III, show that marked losses of the acetone-soluble material occurred in cultures of resin-clearing organisms. The amount of resin lost was high for organisms which produced distinct emulsion clearing, low for *B. subtilis*, which caused indistinct clearing apparent only after several weeks, and was within the experimental error for # 166, a small gram-negative bacterium which did not clear resin emulsions.

The rate of resin loss was always more rapid in media containing beef extract and peptone (Medium II). This result confirms the observations that resin digestion was more rapid in Medium II, as evidenced by the more rapid appearance of clearing of agar plates.

Resin decomposition in liquid media was not complete, but represented a selective removal of certain constituents. Cultures of 1H kept for several months still had an appreciable amount of emulsified resin, although the density of the emulsion was visibly less than that of controls. When the resin on which 1H had acted was made up in Medium I and inoculated with 3HF, *Aspergillus fumigatus*, and *Actinomyces fradii*, no resin clearing occurred, although growth was good. The resin constituents which these organisms decompose were apparently removed by 1H.

SUMMARY

1. The occurrence of resin-decomposing organisms during the retting of guayule shrub by the natural microflora for the purpose of producing improved guayule rubber has been studied.

2. A method is described for detecting resin-decomposing organisms by their ability to cause clearing of resin emulsions suspended in agar.

3. Guayule resins are not appreciably bactericidal.

4. Resin-decomposing organisms isolated include (a) *Aspergillus fumigatus*, (b) *Rhizopus arrhizus*, (c) a member of the Dematiaceae, (d) *Actinomyces fradii*, (e) a species of *Micrococcus*, (f) 3 isolates of the *Bacillus subtilis* group, (g) an unidentified gram-negative rod, (h) *Pseudomonas boreopolis*, and (i) *Achromobacter lacticum*.

5. All these organisms attack some portion of the crude-rubber extract, but the unsaponifiable fraction contains compounds attacked only by *Actinomyces fradii*, the member of the Dematiaceae and the unidentified gram-negative rod. Only the latter two attack the exuded resin.

6. The rate and extent of clearing of resin-agar emulsions by these organisms is correlated with the decomposition of resin in liquid culture, as shown by chemical analysis.

7. The presence of nutrients does not prevent the production of resin-decomposing enzymes, but on the contrary, by increasing the amount of growth, leads to more rapid and extensive resin clearing.

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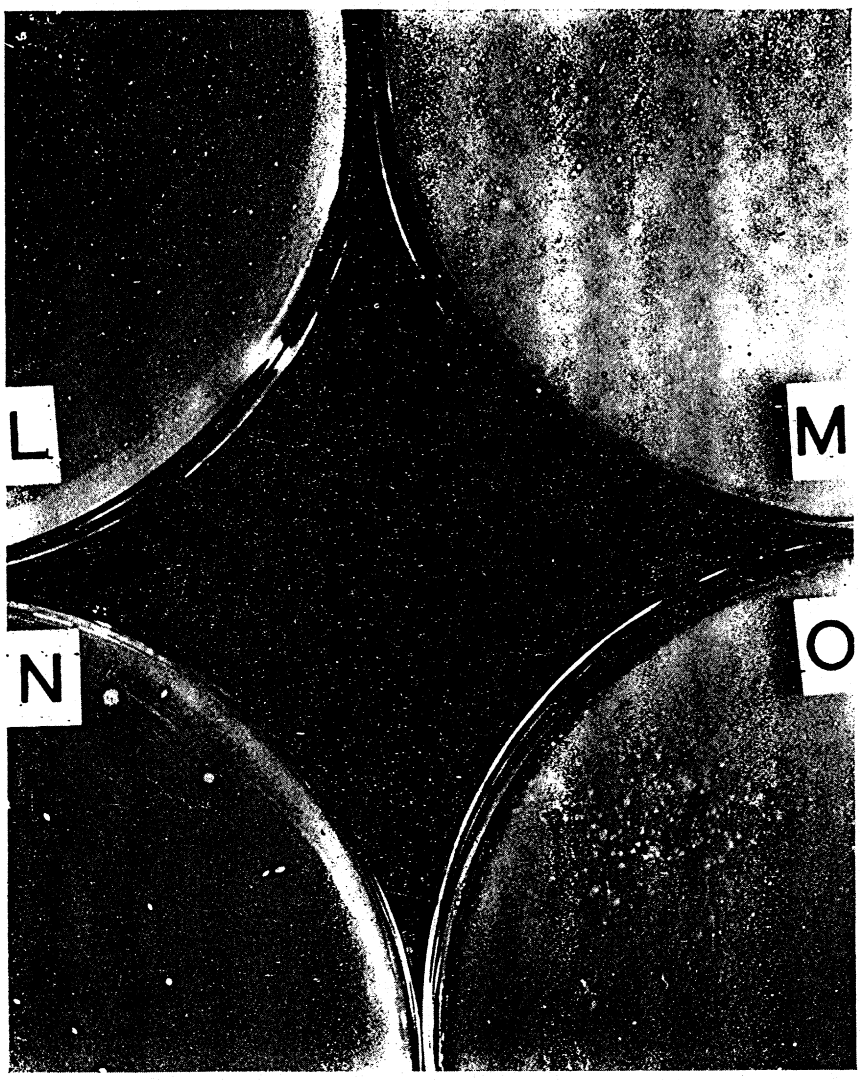

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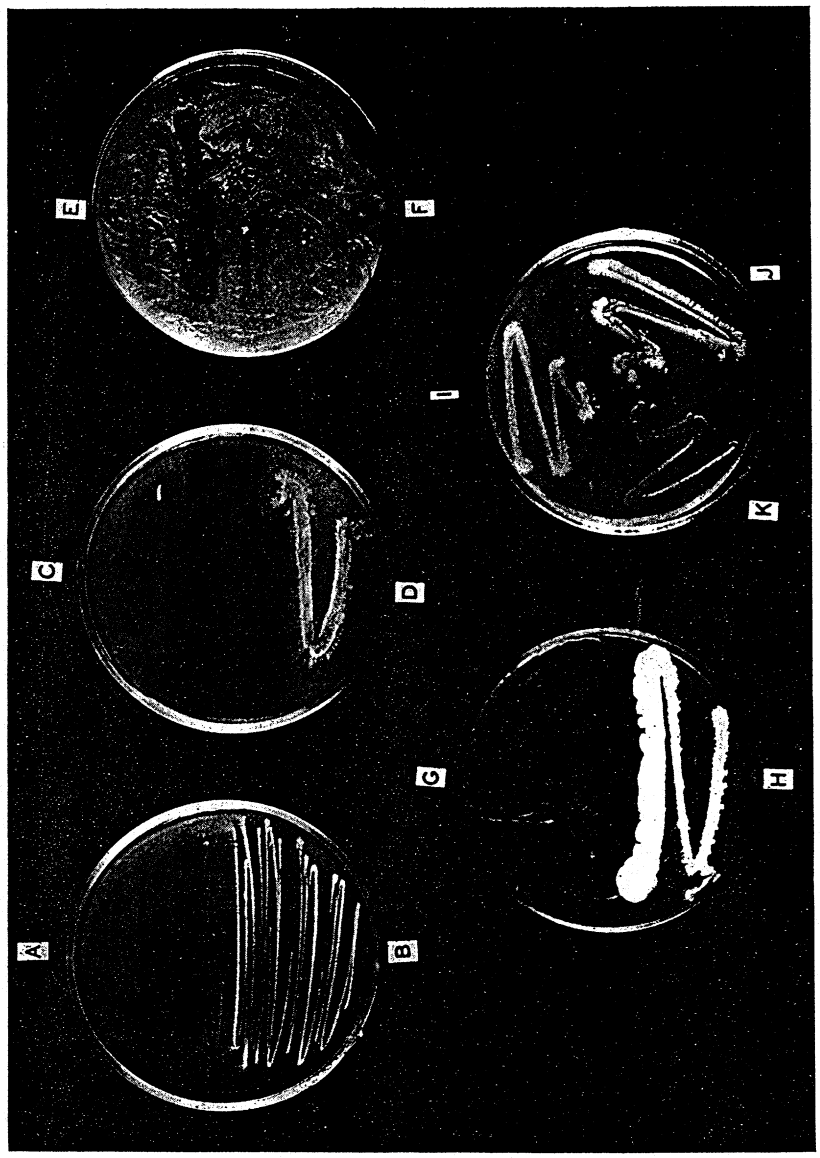
EXPLANATION OF PLATES

Explanation of Plate I (streak cultures) and Plate II (poured plates) showing resin-digesting organisms upon resin agar.

CODE	ORGANISM	MEDIUM	RESIN	COMMENTS
A	1H (unidentified rod)	I	4	Distinct clearing along streaks
B	*Actinomyces fradii*	I	4	No clearing; slight growth
C	Dematiaceae	I	1	Diffuse, pronounced clearing
D	*Actinomyces fradii*	I	1	Slight clearing; fair growth
E	Dematiaceae	I	3	Pronounced diffuse clearing
F	*Bacillus subtilis*	I	3	No growth; no clearing
G	Dematiaceae	II	1	Pronounced diffuse clearing
H	*Actinomyces fradii*	II	1	Distinct clearing around very heavy growth
I	*Achromobacter lacticum*	II	3	Slight clearing at margin of heavy growth (cf. N)
J	*Achromobacter lacticum* (another isolate)	II	3	Slight clearing at margin of heavy growth
K	*Pseudomonas boreopolis*	II	3	Slight clearing at margin of heavy growth (cf. M)
L	1H (unidentified rod)	I	3	Distinct clearing around individual colonies
M	*Pseudomonas boreopolis*	I	3	Distinct clearing around individual colonies
N	*Achromobacter lacticum*	I	3	Distinct clearing around individual colonies
O	*Aspergillus fumigatus*	I	3	Diffuse slight clearing of area around colonies



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